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Relationship of photosynthetic acclimation to changes of Rubisco activity in field-grown winter wheat and barley during growth in elevated carbon dioxide

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Abstract

The responses of photosynthesis, Rubisco activity, Rubisco protein, leaf carbohydrates and total soluble protein to three carbon dioxide treatments were studied in winter wheat [*Triticum aestivum* (L.)] and barley [*Hordeum vulgare* (L.)]. Barley and wheat plants were grown in small field plots during 1995 and 1996 in clear, acrylic chambers (1.2–2.4 m^2) and were provided with continuous carbon dioxide fertilization at concentrations of 350, 525 and 700 μmol mol^{-1}. Photosynthetic rates of barley penultimate leaves and wheat flag leaves measured at growth carbon dioxide concentrations decreased with leaf age in all three CO_2 treatments during 1995 and 1996. Photosynthetic acclimation to elevated CO_2 was observed on seven of eight measurement dates for barley and ten of eleven measurement dates for wheat over both years. Initial Rubisco activity, total soluble protein and Rubisco protein in barley penultimate leaves and wheat flag leaves also decreased with leaf age. Total Rubisco activity was not used because of enzyme degradation. There was a significant CO_2 treatment effect on initial Rubisco activity, total soluble protein and Rubisco protein for wheat in 1995 and 1996 and for barley in 1995. Responses of barley penultimate leaf Rubisco activity and leaf protein concentrations to elevated carbon dioxide were nonsignificant in 1996. A significant CO_2 treatment effect also was detected when means of Rubisco activity, soluble protein and Rubisco protein for wheat flag leaves were combined over harvests and years. These three flag leaf parameters were not significantly different in the 350 and 525 μmol mol^{-1} CO_2 treatments but were decreased during growth in 700 μmol mol^{-1} CO_2 relative to the other two CO_2 treatments. Ratios of photosynthesis at 700 and 350 μmol mol^{-1} were compared to ratios of Rubisco activity at 700 and 350 μmol mol^{-1} using wheat flag leaf data from 1995 and 1996. Regression analysis of these data were linear [$y = 0.586 + 1.103x$ ($r^2 = 0.432$)] and were significant at $P \leqslant 0.05$. This result indicated that photosynthetic acclimation was positively correlated with changes of initial Rubisco activity in wheat flag leaves in response to CO_2 enrichment. Effects of elevated CO_2 on wheat leaf proteins during 1995 and 1996 and on barley during 1995 were consistent with an acceleration of senescence.

Abbreviations: DOY – day of year; Rubisco – ribulose 1,5-bisphosphate carboxylase/oxygenase; Chl – chlorophyll; PFD – photon flux density

Introduction

Changes of biomass in response to CO_2 enrichment have been reported to vary with species. A persistent increase of biomass production during growth in elevated CO_2 was observed for both cotton and sour orange trees in multi-year field studies (Idso and Kimball 1992; Kimball and Mauney 1993). Conversely,

biomass yields of orchard grass and alfalfa were unaffected by CO_2 enrichment in the final two years of a three year study (Bunce 1993, 1995). One explanation for the variable effects of elevated CO_2 on dry matter production was that photosynthetic rates of some C_3 plants were diminished following days to weeks of CO_2 enrichment (Kramer 1981). Thus, the photosynthetic capacity of certain economically important terrestrial plants will have to be modified in order to realize potential biomass gains due to future atmospheric change (Badger 1992; Follett 1993).

The suppression of photosynthetic rates in response to CO_2 enrichment has been associated with decreased Rubisco activity and Rubisco protein levels (Wong 1979; Sage et al. 1989; Yelle et al. 1989). Changes of Rubisco protein during growth in elevated CO_2 have been attributed to carbohydrate accumulation in source tissues and to a subsequent down-regulation of specific genes involved in photosynthesis (Webber et al. 1994). There is substantial indirect evidence that the expression of various photosynthetic genes can be regulated by manipulating cellular carbohydrate levels (Sheen 1990; Krapp et al. 1991; Webber et al. 1994). However, changes of nuclear gene transcripts in field-grown spring wheat plants were weakly correlated with leaf carbohydrate status (Nie et al. 1995). Decreased Rubisco activity in CO_2 enriched plants has not been identified as a causal factor in photosynthetic acclimation (Stitt 1991). A second explanation for the loss of Rubisco protein in elevated compared to ambient CO_2-grown wheat plants was that flowering and senescence occurred more quickly during growth in elevated CO_2 (Sionit et al. 1980; Marc and Gifford 1984). Premature senescence has been attributed to increased photosynthetic rates and to faster rates of development under CO_2 enrichment. Because of the various uncertainties discussed above, additional field CO_2 enrichment studies could help clarify relationships between changes of Rubisco activity and decreased photosynthetic capacity.

The purpose of the current study was to compare photosynthetic responses of winter wheat and barley to CO_2 enrichment under local field conditions. Our hypothesis was that photosynthetic acclimation of both species during growth in elevated CO_2 would be correlated with changes of Rubisco activity and Rubisco protein. Much of what we know about photosynthetic acclimation and its resultant causes has been learned under controlled laboratory conditions using plants grown in pots. Consequently, many of the current hypotheses concerning biochemical causes of photosynthetic acclimation have not been tested in multi-year field studies. Our objectives were to evaluate factors associated with changes of photosynthetic capacity in response to elevated carbon dioxide in the field, and then, use this to assess current hypotheses regarding photosynthetic acclimation.

Materials and methods

Plant materials

Winter wheat (*Triticum aestivum* L. cv. Coker) and barley (*Hordeum vulgare* L. cv. Wyson) were grown in the field at Beltsville, MD, USA, in a uniform Codorus silt loam. Plots were seeded at a rate of 12 g m^2 in October of 1994 and 1995 using 18 cm row spacings. Each chamber contained two border and two experimental rows. All plots were fertilized with 10/10/10 (N/P/K) and were limed before planting as described previously (Bunce 1993). Plants were grown in open-topped acrylic chambers covering 1.2 (wheat) and 2.4 (barley) m^2 land area. Carbon dioxide treatments were ambient (350 ± 50 μmol mol^{-1} measured during the daytime), ambient plus 175 ± 50 μmol mol^{-1} (525 μmol mol^{-1}) and ambient plus 350 ± 50 (700 μmol mol^{-1}). Three chambers of each species and CO_2 treatment were established each year and CO_2 treatments were provided continuously from planting. Air flow rate through the chambers was either 6 m^3 min^{-1} (wheat) or 12 m^3 min^{-1} (barley). Mixing fans provided an air speed of 1.5 m s^{-1} at the top of the crop canopy. Plants received natural precipitation but did not experience significant soil moisture deficits. Mean 24 h temperatures within the plots exceeded the outside air temperature by 1 °C on average and chambers used for the three CO_2 treatments differed less than 0.5 °C. Crops received about 90% of the outside photosynthetically active radiation.

Photosynthesis measurements

Single leaf photosynthetic rates were determined at 7 to 10 d intervals using a portable, open photosynthesis system with CO_2 control (CIRAS-1, PP Systems, Haverhill, MA, USA). Measurements were initiated during April of 1995 and 1996 when barley plants were in the late boot stage of development (i.e. pre-anthesis) and were terminated when grain filling was completed and the upper canopy leaves had senesced. Measurements were performed on wheat flag leaves

and barley penultimate leaves in full mid-day sunlight, when the incident PFD exceeded 1500 μmol m^{-2} s^{-1}. Air temperatures were ambient and varied from 15 to 37 °C on the selected measurement dates. Mean leaf temperatures did not differ by more than 1 °C between CO_2 treatments on a given day and gas exchange measurements were usually initiated and completed within 1 h. The order in which the two species was measured varied between days. Mean leaf temperature differed between species by up to 3 °C on a given measurement date. Photosynthetic rates were determined at carbon dioxide concentrations used for plant growth and photosynthetic rates of leaves in the low carbon dioxide treatment also were measured at 700 μmol mol^{-1} CO_2. Photosynthesis values were based on the average of at least two leaf rate measurements for each chamber (n = 6 per treatment). Photosynthetic acclimation was defined in two ways. First, acclimation to elevated CO_2 was measured as a significantly lower assimilation rate for leaves developed at 700 compared to leaves developed at 350 μmol mol^{-1} , when using a measurement CO_2concentration of 700 μmol mol^{-1}. Photosynthetic acclimation also was observed when the assimilation rate of plants grown and measured in the elevated CO_2 treatment did not differ significantly from that of plants grown and measured in the ambient CO_2 treatment.

Biochemical measurements

Leaf samples were harvested for Rubisco activity, Rubisco protein and carbohydrate measurements at times corresponding to CO_2 exchange rate measurements. Leaf segments of 2 (Rubisco activity) 4 (Rubisco protein) and 6 cm (carbohydrates) length were removed from central portions of the lamina of wheat flag leaves and barley penultimate leaves. Segments were collected from fully expanded upper canopy leaves exposed to full sunlight. Areas were calculated before excision by measuring widths at the center of each leaf segment. Leaf tissue was quickly transferred to liquid N_2 contained in envelopes made of Al foil. Samples were transported from the field in liquid N_2 and were stored at -80 °C until use. Values were the average of one leaf sample per chamber.

Initial and total Rubisco activity measurements were performed using 2 cm leaf segments as described previously (Perchorowicz et al. 1981; Sicher et al. 1994). Radiochemical assays were initiated with 25 μl sample and were terminated after 30 s at 25 °C with 0.2 ml of 0.5 N HCl. An aliquot of each extract was used to measure soluble protein (Bradford 1976). Total soluble protein was estimated from standard curves prepared with bovine serum albumin. Rubisco protein was measured according to the procedure of Makino et al. (1986). Leaf segments (4 cm) were homogenized with 2 ml extraction buffer containing 50 mM Bicine-NaOH, pH 8.6, 20 mM M gCl_2, 10 mM $NaHCO_3$ and 40 mM 2-mercaptoethanol. After a brief centrifugation step, leaf proteins were separated by SDS-PAGE. Gels were stained with Coomassie brillant blue-R, and after destaining, bands corresponding to the large subunit of Rubisco (55 kD) were excised and quantified as described previously (Sicher et al. 1994). Rubisco acclimation was defined as a decrease of Rubisco activity or Rubisco protein in response to CO_2 enrichment.

Samples for carbohydrate analyses were extracted at 0 °C with 4 ml of methanol: chloroform: 50 mM Tris base (8:3:1) in ground glass tissue homogenizers. The homogenates were centrifuged at 3000 g for 5 min in 15 ml conical centrifuge tubes and the pellets were re-extracted with 1 ml of 80% methanol. Supernatants were combined and the solvent extracts were fractionated by phase partitioning. Leaf Chl concentrations were determined using aliquots of the organic phase. Starch in the pellet fractions was measured after digestion with amyloglucosidase and α-amylase (Sicher et al. 1994). The aqueous-alcohol fractions were evaporated to remove methanol and sucrose was hydrolyzed with acid invertase at pH 4.5. Glucose liberated from starch or sucrose was measured in coupled enzyme assays according to Bergmeyer et al. (1965). Values are expressed as hexose equivalents after correcting for hydrolysis.

Statistical analysis

Results for wheat and barley were analyzed separately by an analysis of variance procedure (SuperANOVA, Abacus Concepts, Berkeley, CA, USA). Data for each year were analyzed for significant differences by date and by treatment. Each year's results were combined if the treatment by date interaction was nonsignificant and significant differences were for $P \leqslant 0.05$. Slopes obtained by linear regression were tested by analysis of covariance.

Results

Single leaf photosynthetic rates

Single leaf photosynthetic rates of barley and wheat

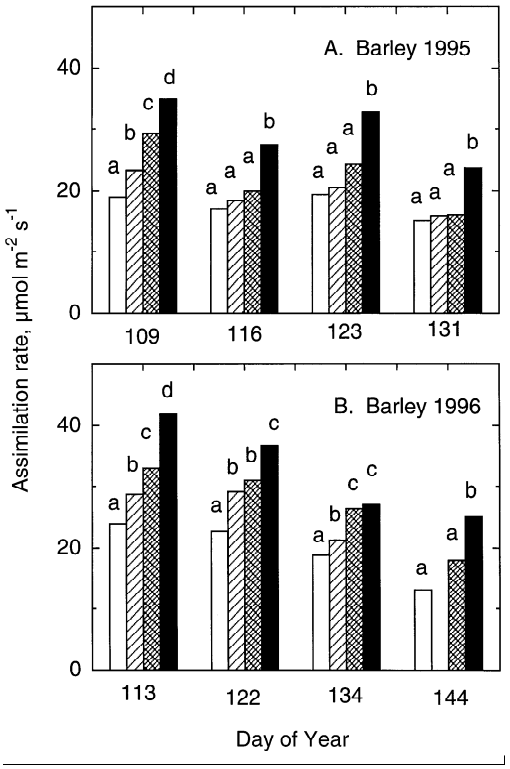

Figure 1. Photosynthetic rates of barley penultimate leaves in response to three CO_2 treatments. Photosynthesis rates for each date are reported for measurements performed at growth CO_2 concentrations of 350 μmol mol^{-1} (bar without fill); 525 μmol mol^{-1} (bar with single hatch) and 700 μmol mol^{-1} (bar with cross hatch), respectively. The fourth photosynthetic rate (bar with solid fill) was for the leaves in the ambient CO_2 treatment measured at 700 μmol mol^{-1}. Panels A and B were for data collected in 1995 and 1996, respectively. Letters above bars denote significant differences at $P \leqslant 0.05$.

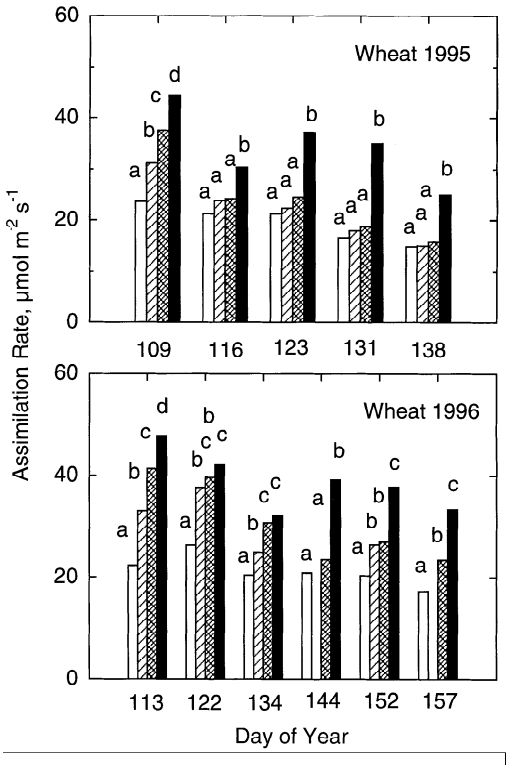

Figure 2. Photosynthetic rates of wheat flag leaves in response to three CO_2 treatments. Experimental details were as in Figure 1.

during 1995 and 1996 are shown in Figures 1 and 2, respectively. A significant treatment by date interaction for single leaf photosynthetic rates was observed for barley in 1995 and 1996 (Figure 1A and 1B) and for wheat in 1996 (Figure 2B). Therefore, results for each measurement date were analyzed separately. Maximal photosynthetic rates of both species were observed on the first measurement dates of 1995 and 1996. There also was a significant treatment effect of CO_2 on single leaf photosynthetic rates of barley and wheat on these dates. Net photosynthetic rates of both species, irrespective of CO_2 treatment, decreased with leaf age. An enhancement of photosynthesis in the high compared to the low CO_2 treatment was not observed for barley on the last measuring dates of 1995 and 1996. Photosynthetic rates of wheat plants in the high and low CO_2 treatments also did not differ on the last measurement date of 1995. Photosynthetic acclimation to elevated CO_2 was observed for both wheat and barley at almost every measurement date. Photosynthetic acclimation was observed in the barley plots at seven of eight measurement dates performed over two years. Similarly, photosynthetic acclimation was observed in wheat at 10 of 11 measurement dates in 1995 and 1996.

Rubisco activity

Rubisco activity in barley penultimate leaves was measured in all three CO_2 treatments on four dates in 1995 and on five dates in 1996 (Figures 3A and 3C). In comparison, Rubisco activity in wheat flag leaves was measured on five dates in 1995 and seven dates in 1996 (Figures 3B and 3D). This reflected the fact that barley matured more quickly than wheat and that maturation of both species was earlier in 1995 in comparison to 1996. Initial and total Rubisco activities were measured on all wheat and barley samples, although percent activation, measured as the ratio of initial to total enzyme activity, usually was equal to or exceeded

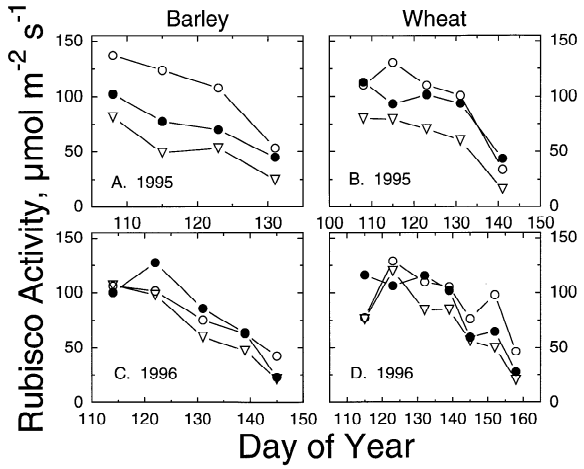

Figure 3. Rubisco activity of barley penultimate leaves and wheat flag leaves in response to three CO_2 treatments. Initial Rubisco activity was measured at 25 °C and pH 8.0 using leaf samples of plants grown in the field at 350 (○) 525 (●) and 700 (▽) μmol mol^{-1} CO_2. Panels were for barley (A, C) and wheat (B, D) for data collected in 1995 (A, B) and 1996 (C, D), respectively.

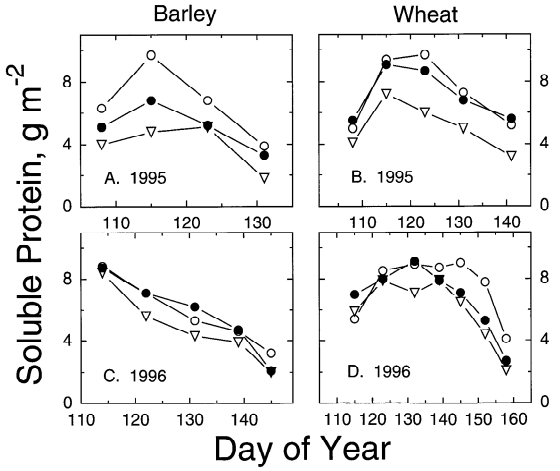

Figure 4. Total soluble protein concentrations of barley penultimate leaves and of wheat flag leaves in response to three CO_2 treatments. Experimental details and symbols were as in Figure 3.

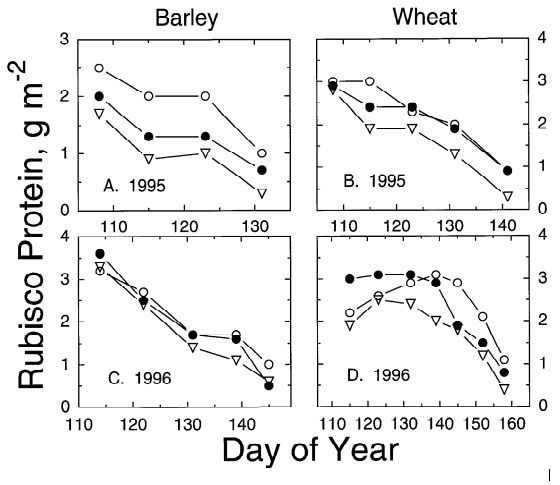

Figure 5. Rubisco protein concentrations of barley penultimate leaves and of wheat flag leaves in response to three CO_2 treatments. Experimental details and symbols were as in Figure 3.

100%. This indicated that, in spite of the precaution of incubating the enzyme for both species at 0 °C, Rubisco activity was degraded during activation with CO_2 and Mg^{2+}. Therefore, only initial Rubisco activity measurements were reported in this study. In agreement with the photosynthesis data described above, initial Rubisco activity for both species and during both years was maximal on the first or second sampling date and decreased with leaf age.

There was a significant CO_2 treatment effect on Rubisco activity for wheat samples in both 1995 and 1996, when means for each year were compared across all harvest dates (Table 1). Treatment by harvest date interactions for both years were nonsignificant enabling these data to be combined for comparison. Combined data for both years also differed significantly ($P \leqslant 0.001$) and showed that initial Rubisco activity of wheat in the high CO_2 treatment was decreased relative to the medium and low CO_2 treatments. Rubisco activities of wheat samples from the low and medium CO_2 treatments did not differ. Rubisco activity data for barley during the first and second years of the study were analyzed separately. This was necessary because, unlike the above analysis for wheat, significant treatment effects of CO_2 enrichment were observed for barley in 1995 but not in 1996. Averaged over all four harvests in 1995, Rubisco activity of barley plants in the high CO_2 treatment was half that observed in the low CO_2 treatment.

Leaf proteins

Two qualitative patterns of protein changes were observed in the current study (Figures 4 and 5). In the first pattern, soluble protein and Rubisco protein concentrations were maximal on the first harvest and then protein levels decreased with increasing leaf age. This pattern was observed for barley soluble protein in 1996 (Figure 4C) and for barley Rubisco protein in 1995 and

Table 1. Means and analysis of variance of growth carbon dioxide effects on Rubisco activities from wheat and barley leaves. Data are means ± SE for all measurements shown in Figure 3. Enzyme rates are in μmol m^{-2} s^{-1}. Values not followed by the same letter differ at $P \leqslant 0.05$

Species	Growth CO_2	1995	1996	Both years
Wheat	350	113 ± 5.5	92 ± 6.7	99 ± 5.0 a
	525	100 ± 4.5	85 ± 7.9	90 ± 5.4 a
	700	72 ± 6.5	70 ± 7.1	71 ± 5.0 b
Anova results:				
Significance		*P*	*P*	*P*
Treatment		0.0001	0.0013	0.0006
Harvest		0.2749	0.0001	
Treatment * harvest		0.7316	0.0989	
Barley	350	106 ± 11	78 ± 7	
	525	74 ± 6		80 ± 10
	700	52 ± 7	67 ± 9	
Anova results:				
Significance		*P*	*P*	
Treatment		0.0001	0.0618	
Harvest		0.0001	0.0001	
Treatment * harvest		0.2691	0.3153	

1996 (Figure 5A and 5C). A near linear loss of Rubisco protein also was observed in wheat flag leaves during 1995 (Figure 5B). In the second pattern, total soluble protein and Rubisco protein concentrations were low initially, increased to maxima during early grain-filling and then protein levels decreased through the final harvest. Soluble leaf proteins in barley during 1995 (Figure 4A) and in wheat during 1995 and 1996 (Figure 4B and 4D) followed the latter pattern. Rubisco protein in wheat during 1996 also increased slightly with leaf age before subsequent mobilization was observed (Figure 5D).

Effects of CO_2 enrichment on soluble protein and on Rubisco protein levels in barley and wheat leaves were in broad agreement with data discussed above for Rubisco activity. Means for separate years were compared over harvest dates and significant CO_2 treatment effects on soluble protein and on Rubisco protein were observed for wheat samples in 1995 and 1996 (Table 2 and 3). Significant CO_2 treatment effects also were observed for wheat soluble protein and Rubisco protein when data for both years were combined. These combined data for wheat flag leaves showed that soluble protein and Rubisco protein did not differ between the 350 and 525 μmol mol^{-1} CO_2 treatments. However, soluble protein and Rubisco protein levels were decreased in the 700 compared to either the 350 or 525 μmol mol^{-1} CO_2 treatments. Effects of CO_2 enrichment on barley soluble protein and Rubisco protein varied between years. Both soluble protein and Rubisco protein concentrations in barley penultimate leaves were significantly decreased by CO_2 enrichment in 1995. However, the same CO_2 treatment was nonsignificant in 1996.

Carbohydrates and Chl

Wheat and barley were sampled for carbohydrate and Chl measurements during the 1996 growing season. In general, the pattern of carbohydrate storage and responses to CO_2 enrichment differed for wheat flag leaves and barley penultimate leaves (Figures 6 and 7). Starch, sucrose and glucose were low in barley penultimate leaves on DOY 114 and then non-structural carbohydrate levels were either unchanged or increased with leaf age through the final harvest (Figure 6A, 6B and 6C). Mean starch and sucrose levels, but not glucose concentrations, were greater ($P \leq 0.05$) in barley leaf samples grown at 700 compared to 350 μmol $mol^{-1}CO_2$ treatments, when averaged over all har-

Table 2. Means and analysis of variance of growth carbon dioxide effects on soluble protein concentrations in wheat and barley leaves. Data are means ± SE for all measurements shown in Figure 4. Protein concentrations are in g m^{-2}. Values not followed by the same letter differ at $P \leqslant 0.05$

Species	Growth CO_2	1995	1996	Both years
Wheat	350	7.8 ± 0.8	7.5 ± 0.5	7.6 ± 0.4 a
	525	7.5 ± 0.6	6.7 ± 0.5	7.0 ± 0.4 a
	700	5.6 ± 0.6	6.0 + 0.5	5.8 ± 0.4 b
Anova results:				
Significance		*P*	*P*	*P*
Treatment		0.0089	0.0005	0.0044
Harvest		0.0003	0.0001	
Treatment * harvest		0.8959	0.1551	
Barley	350	6.8 ± 0.8	5.8 ± 0.6	
	525	5.1 ± 0.5	5.7 ± 0.7	
	700	4.0 ± 0.4	4.8 ± 0.6	
Anova results:				
Significance		*P*	*P*	
Treatment		0.0001	0.0618	
Harvest		0.0001	0.0001	
Treatment * harvest		0.2691	0.3153	

Table 3. Means and analysis of variance of growth carbon dioxide effects on Rubisco protein concentrations in wheat and barley leaves. Data are means ± SE for all measurements shown in Figures 5. Protein concentrations are in g m^{-2}. Values not followed by the same letter differ at $P \leqslant 0.05$

Species	Growth CO_2	1995	1996	Both years
Wheat	350	2.6 ± 0.2	2.4 ± 0.2	2.5 ± 0.1 a
	525	2.4 ± 0.2	2.3 ± 0.2	2.3 ± 0.1 a
	700	2.0 ± 0.2	1.8 ± 0.2	1.8 ± 0.1 b
Anova results:				
Significance		*P*	*P*	*P*
Treatment		0.0021	0.0001	0.0019
Harvest		0.0001	0.0001	
Treatment * harvest		0.3783	0.1631	
Barley	350	1.9 ± 0.2	2.1 ± 0.3	
	525	1.3 ± 0.2	2.0 ± 0.3	
	700	1.0 ± 0.2	1.7 ± 0.3	
Anova results:				
Significance		*P*	*P*	
Treatment		0.0001	0.2033	
Harvest		0.0001	0.0001	
Treatment * harvest		0.8058	0.8656	

Table 4. Means of growth carbon dioxide effects on carbohydrate and Chl concentrations in field-grown wheat and barley leaves. Data are means ± SE for measurements shown in Figures 6 and 7. Starch and sucrose (g m^{-2}) are expressed as hexose equivalents. Values not followed by the same letter differ at $P \leqslant 0.05$

Parameter	Growth CO_2	Wheat	Barley
		mmol m^{-2}	
Glucose	350	1.72 ± 0.2 a	0.21 ± 0.1 a
	525	2.32 ± 0.3 a	0.52 ± 0.1 a
	700	1.81 ± 0.2 a	0.42 ± 0.1 a
Sucrose	350	12.3 ± 1.0 a	4.10 ± 0.9 a
	525	13.0 ± 1.1 a	5.27 ± 0.8 ab
	700	13.6 + 1.1 a	8.42 ± 1.1 b
Starch	350	4.1 ± 0.3 a	2.0 ± 0.1 a
	525	5.0 ± 0.5 ab	2.6 ± 0.3 ab
	700	5.3 ± 0.4 b	3.1 ± 0.4 b
		g m^{-2}	
Chl	350	0.43 ± 0.04 a	0.42 ± 0.02 a
	525	0.38 ± 0.02 ab	0.40 ± 0.03 a
	700	0.35 ± 0.02 b	0.39 ± 0.02 a

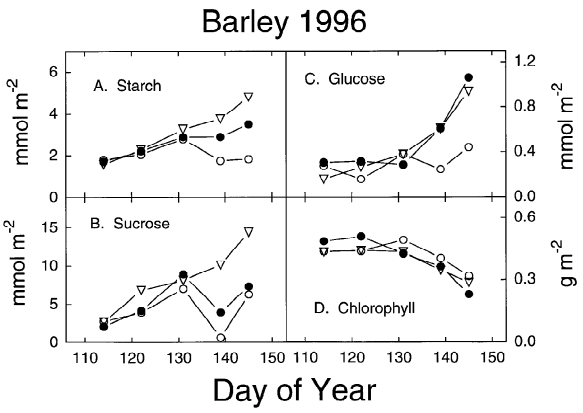

Figure 6. Changes of carbohydrates and Chl in barley penultimate leaves. Leaf starch (panel A), sucrose (panel B), glucose (panel C) and Chl (panel D) were measured at indicated times in response to three CO_2 treatments; 350 (○), 525 (●) and 700 (▽) μmol mol^{-1} CO_2.

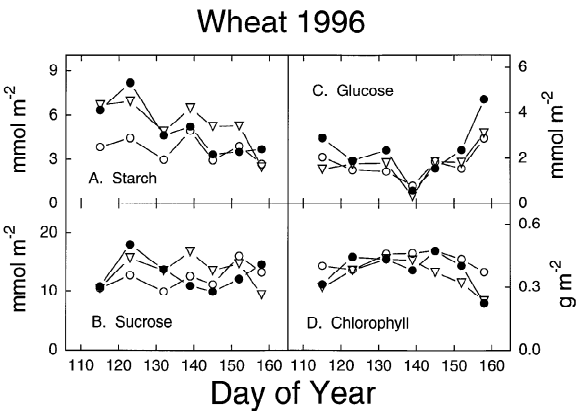

Figure 7. Changes of carbohydrates and Chl in wheat flag leaves. Experimental details were as in Figure 6.

vests (Table 4). This was due to the accumulation of storage carbohydrates in barley leaves in response to CO_2 enrichment on the final two harvests.

Starch levels in wheat flag leaves were 3.9 mmol m^{-2} under the ambient CO_2 treatment and little change was observed over the remainder of the growing season (Figure 7A). In comparison, starch levels in samples from the two elevated CO_2 treatments were greater than 6 mmol m^{-2} on the first sampling and these initial starch levels decreased with leaf age. Starch concentrations differed ($P \leqslant 0.05$) in the 350 and 700 μmol mol^{-1} CO_2 treatments, when data for flag leaf samples were averaged over all harvests during 1996. In contrast to starch, sucrose concentrations in wheat flag leaves were unaffected by CO_2 enrichment and no obvious effects of leaf age were observed

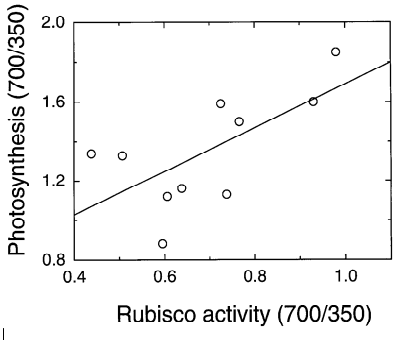

Figure 8. Linear regression of photosynthesis and initial Rubisco activity of wheat flag leaves under high and low CO_2 treatments. Ratios of photosynthesis measured at 700 and 350 μmol mol^{-1} CO_2 and of initial Rubisco activity at 700 and 350 μmol mol^{-1} CO_2 were compared using wheat data for 1995 and 1996 as shown in Figures 2 and 3.

on sucrose (Figure 7B). Mean glucose concentrations were 4- to 8-fold greater in wheat flag leaves than in barley penultimate leaves (Table 4). The pattern of glucose storage in leaf blades of both species was similar and an accumulation of hexose was observed on the last harvest (Figure 7C). Glucose concentrations in wheat flag leaves were variable because of changing environmental conditions in the field. In agreement with results for sucrose, mean glucose levels in wheat flag leaves did not differ among CO_2 treatments when averaged over all harvests.

Changes of Chl in barley penultimate leaves and wheat flag leaves in response to elevated CO_2 treatment were qualitatively similar to changes of soluble protein concentration (Figures 6C and 7C). Wheat flag leaf Chl levels were lower ($P \leqslant 0.05$) in the 700 μmol mol^{-1} compared to the 350 μmol mol^{-1} CO_2 treatment, when averaged over 7 harvests. Conversely, there were no signifcant treatment effects of CO_2 on leaf Chl levels in barley during 1996.

Photosynthesis and Rubisco

It was of interest to determine if a correlation could be constructed between changes of photosynthesis and Rubisco activity when comparing plants grown in ambient and elevated CO_2 treatments. The ratio of photosynthesis rates measured in the high and low CO_2 treatments was compared by date to similar ratios of initial Rubisco activity for wheat flag leaves (Figure 8). These data were analyed by linear regression and were for combined photosynthesis and Rubisco activity measurements during 1995 and 1996. The regression fitted the formula $y = 0.586 + 1.103x$ ($r^2 = 0.432$). The regression was significant ($P \leqslant 0.05$) even though photosynthesis measurements were performed at different ambient temperatures on different days. The regression indicated that photosynthetic rates of plants grown and measured at 700 μmol mol^{-1} CO_2 equalled those of plants grown and measured at 350 μmol mol^{-1} CO_2, when Rubisco activity in the former was reduced by 60%. Similar regressions constructed for barley photosynthesis and Rubisco measurements were nonsignificant, largely because Rubisco acclimation was not observed in 1996. There also were insufficient data points to obtain a significant regression between barley photosynthetic rates and Rubisco activity measured only during 1995.

Discussion

Our hypothesis stated that photosynthetic acclimation of wheat and barley leaves should be correlated with Rubisco acclimation during growth in elevated CO_2. Current results for wheat flag leaves obtained over two years were in support of this hypothesis. Results for barley differed between years, so the summary data were inconclusive. It should be noted that results for wheat were based on comparisons of plants grown in the 700 and 350 μmol mol^{-1} CO_2 treatments. This was necessary because Rubisco acclimation was not observed for wheat grown at 525 μmol mol^{-1} CO_2, when data were summed over harvests in the present study. Nie et al. (1995) previously studied changes of Rubisco protein in field-grown spring wheat in response to CO_2 enrichment. In contrast to current findings, these authors observed decreased Rubisco protein levels in wheat flag leaves grown under 550 μmol mol^{-1} CO_2. It is possible that effects of the medium CO_2 treatment on Rubisco protein levels differed between these two studies because of the higher ambient light levels that occur in the Southwestern U.S.

Based on various lines of evidence Stitt and Schulze (1994) concluded that the control of photosynthesis by Rubisco activity was minimal at low PFD ($<$ 1000 μmol m^{-2} s^{-1}) and was high when PFD and CO_2 were near-saturating. Although field conditions vary with time and location, the relatively high ambient light levels in this study suggested that Rubisco activity had the potential to limit photosynthetic rates in wheat flag leaves. In support of this conclusion, the regression data in Figure 8 showed a correlation between decreased Rubisco activity and down regu-

lation of the net stimulation of photosynthesis during growth in elevated CO_2. This linear relationship between changes of photosynthesis and Rubisco activity suggested that changes of Rubisco activity in wheat flag leaves in response to CO_2 enrichment resulted in decreased photosynthetic capacity.

As noted above, comparisons of photosynthetic acclimation and Rubisco acclimation using barley were unsuccessful. This was primarily because Rubisco acclimation in response to CO_2 enrichment was not detected in penultimate leaves during 1996. It should be pointed out that photosynthetic acclimation in barley was observed both years and at almost every measurement date. Therefore, photosynthetic aclimation was observed in the absence of Rubisco acclimation. There are two possible explanations for this observation. First, photosynthesis measurements are far less variable than Rubisco assays. Because of this, small differences of photosynthesis due to CO_2 treatment would be more easily detected than changes of Rubisco activity. Second, photosynthetic acclimation would occur in the absence of Rubisco acclimation, if factors other than Rubisco were responsible for decreased photosynthetic capacity. For example, photosynthetic rates of soybean leaves also have been reported to decrease during growth in elevated CO_2 with no detectable changes of Rubisco activity or of Rubisco protein concentration (Campbell et al. 1988; Sicher et al. 1995).

The observed effects of CO_2 enrichment on wheat were consistent with accelerated development and premature sensecence as reported previously (Sionit et al. 1980; Marc and Gifford 1984). Sionit et al. (1980) observed that anthesis of wheat was advanced by 1 to 2 d and senescence by 7 d in response to CO_2 enrichment. Anthesis also was 1 to 2 d earlier in field-grown spring wheat exposed to 550 compared to 350 μmol mol^{-1} CO_2 (Nie et al. 1994) and similar effects of CO_2 enrichment on anthesis were observed in the current study (J.A. Bunce, unpublished). Mean Rubisco protein concentrations in wheat and barley leaves comprised 31 – 36% of total soluble protein, respectively, in the current study. This ratio was maintained across years and CO_2 treatments and was so constant that soluble protein measurements could serve as a proxy for Rubisco protein and Rubisco activity. Wherever responses to elevated CO_2 were detected, decreases of soluble protein and Rubisco protein were concomitantly earlier in the high compared to the low CO_2 treatment. Decreased Chl levels in wheat flag leaves in response to elevated CO_2 treatment during 1996 also were correlated with decreased soluble protein and Rubisco protein . Moreover, elevated CO_2 did not affect either Chl or leaf protein levels in barley penultimate leaves during 1996. Therefore, one possible explanation for the down regulation of Rubisco activity and of photosynthesis in CO_2 enriched wheat and barley was that Rubisco protein decreased in response to premature senescence that was caused by accelerated rates of development and maturation.

Genes transcribing Rubisco and other photosynthetic proteins can be down regulated by elevated cellular carbohydrate levels (Stitt 1991; Krapp et al. 1991). Evidence that transcripts for the large and small subunits of Rubisco were down regulated in response to CO_2 enrichment previously was observed for wheat flag leaves but was not observed for other leaves on the plant sampled during vegetative growth (Nie et al. 1994, 1995). At the present time, mechanisms linking carbohydrate accumulation to the regulation of gene expression remain unidentified, although current evidence suggests that cellular carbohydrate concentrations are detected by hexokinase activity (Sheen 1994). In the present study, no direct correlation between leaf carbohydrate levels and photosynthetic acclimation was observed for either species. Overall, leaf carbohydrate levels in wheat and barley leaves were low and were only moderately affected by elevated CO_2 treatment. Rogers et al. (1993) also reported that wheat leaves accumulated very little of the excess carbohydrate produced at elevated CO_2 and attributed this to increased tillering. Furthermore, Nie et al. (1994) reported that the total nonstructural content of wheat flag leaves only increased by about 10% over a single photoperiod in elevated compared to ambient CO_2-grown plants. These findings for small grains crops were surprising, since it is generally assumed that doubling the atmospheric CO_2 concentration will have a large impact on the source/sink balance of the plant (Stitt 1991).

Starch levels were elevated in wheat flag leaves of the current study, when comparing the low and high CO_2 treatments. Effects of CO_2 enrichment on starch were most obvious early in leaf development. Starch is an insoluble polyglucan that is synthesized exclusively in the plastid. Therefore, it is not obvious how elevated starch levels could directly affect gene regulation. The soluble storage carbohydrates, sucrose and glucose, were either unaffected by CO_2 enrichment or a buildup was only observed in the terminal stages of leaf development. There are several reasons why single leaf carbohydrate measurements may not be a valid

predictor of carbohydrate regulated gene expression using field-grown wheat and barley exposed to elevated CO_2. First, CO_2 is only one of several environmental factors that affects leaf non-structural carbohydrate concentrations. Second, the lamina of cereal leaves is not an important storage tissue for carbohydrates in comparison to the leaf sheath, stem and culms (Austin and Edrich 1975). Third, storage carbohydrates are compartmentalized in subcellular organelles, so that the cytoplasmic carbohydrate concentrations, which are of primary interest, are uncertain. Lastly, it will be difficult to demonstrate that carbohydrate regulation of gene expression can be adequately tested using cereal crops where developmental effects of CO_2 enrichment are pronounced.

In conclusion, we have observed photosynthetic acclimation to elevated CO_2 for both wheat and barley during consecutive years of a two-year field study. Combined over all measurement dates, photosynthetic acclimation for wheat flag leaves was linearly correlated with Rubisco acclimation, when two year's data for plants grown at 700 μmol mol^{-1} and at 350 μmol mol^{-1} CO_2 were compared. A similar comparison for barley penultimate leaves was inconclusive, because Rubisco acclimation was variable over years. In general, Rubisco acclimation in wheat was correlated with changes of soluble protein, Rubisco protein and Chl. Present results suggested that accelerated development and premature senescence were the primary factors affecting Rubisco activity in response to CO_2 enrichment.

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References

Austin RB and Edrich J (1975) Effects of ear removal on photosynthesis, carbohydrate accumulation and on the distribution of assimilated ^{14}C in wheat. Ann Bot 39: 141–151

Badger M (1992) Manipulating agricultural plants for a future high CO_2 environment. Aust J Bot 40: 421–429

Bowes G (1991) Growth at elevated CO_2: Photosynthetic responses mediated through Rubisco. Plant Cell Environ 14: 795–806

Bradford MM (1976) A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248–254

Bunce JA (1993) Growth, survival, competition, and canopy carbon dioxide and water vapor exchange of first year alfalfa at an elevated CO_2 concentration. Photosynthetica 29: 557–565

Bunce JA (1995) Long-term growth of alfalfa and orchard grass plots at elevated carbon dioxide. J Biogeography 22: 341–348

Bergmeyer HU, Brent E, Schmidt F and Stock H (1974) D-Glucose. Determination with hexokinase and glucose 6-phosphate dehydrogenase. In: Bergmeyer HU (ed) Methods of Enzymatic Analysis, Vol 3, 2nd ed, pp 1196-1198, Academic Press, New York

Campbell WJ, Allen LH, Jr and Bowes g (1988) Effects of CO_2 concentration on Rubisco activity, amount, and photosynthesis in soybean leaves. Plant Physiol 67: 1007–1010

Follett RF (1993) Global climate change, US agriculture, and carbon dioxide. J Prod Agric 6: 181–190

Idso S and Kimball BA (1992) Effects of atmospheric CO_2 enrichment on photosynthesis, respiration, and growth of sour orange trees. Plant Physiol 99: 341–343

Kimball BA and Mauney JR (1993) Response of cotton to varying CO_2, irrigation and nitrogen: yield and growth. Agron J 85: 706–712

Kramer PJ (1981) Carbon dioxide concentration, photosynthesis, and dry matter production. BioScience 31: 29–33

Krapp A, Quick WP, Stitt M (1991) Ribulose-1,5-bisphosphate carboxylase oxygenase, other Calvin cycle enzymes, and chlorophyll decrease when glucose is supplied to mature spinach leaves via the transpiration stream. Planta 186: 58–69

Makino A, Mae T and Ohira K (1986) Colorimetric measurement of proteins stained with Coomassie Brillant Blue R on sodium dodecylsulfate polyacrylamide gel electrophoresis by eluting with formamide. Agric Biol Chem (Tokyo) 50: 1911–1912

Nie GY, Long SP, Garcia RL, Kimball BA, Lamporte RL, Pinter PJ, Jr, Wall GW and Webber AN (1995) Effects of free-air CO_2 enrichment on the development of the photosynthetic apparatus in wheat, as indicated by changes in leaf proteins. Plant Cell Environ 18: 855–864

Perchorowicz JT, Raynes DA and Jensen RG (1981) Light limitation of photosynthesis and activation of ribulose bisphosphate carboxylase in wheat seedlings. Proc Natl Acad Sci (USA) 78: 2985–2989

Rogers GS, Payne L, Milham P and Conroy J (1993) Nitrogen and phosphorous requirements of cotton and wheat under changing CO_2 concentrations. Plant and Soil 155/156: 231–234

Sage RF, Sharkey TD and Seemann JR (1989) Acclimation of photosynthesis to elevated CO_2 in five C_3 species. Plant Physiol 89: 590–596

Sheen J (1990) Metabolic repression of transcription in higher plants. Plant Cell 2: 1027–1038

Sheen J (1994) Feedback control of gene expression. Photosynth Res 39: 427–438

Sicher RC, Kremer DF and Bunce JA (1995) Photosynthetic acclimation and photosynthate partitioning in soybean leaves in response to carbon dioxide enrichment. Photosynth Res 46: 409–417

Sicher RC, Kremer DF and Rodermel SR (1994) Photosynthetic acclimation to elevated CO_2 occurs in transformed tobacco with decreased ribulose-1,5-bisphosphate carboxylase/oxygenase content. Plant Physiol 104: 409–415

Sionit N, Hellmers H and Strain BR (1980) Growth and yield of wheat under CO_2 enrichment and water stress. Crop Sci 20: 687–690

Stitt M (1991) Rising CO_2 levels and their potential significance for carbon flow through photosynthesis. Plant Cell Environ 14: 741–762

Stitt M and Schulze D 1994 Does Rubisco control the rate of photosynthesis and plant growth? An excercise in molecular ecophysiology. Plant Cell Environ 17: 465–487

Webber AN, Nie G-Y and Long SP (1994) Acclimation of photosynthetic proteins to rising atmospheric CO_2. Photosynth Res 39: 413–425

Wong SC (1979) Elevated atmospheric partial pressure of CO_2 and plant growth. I. Interactions of nitrogen nutrition and photosynthetic capacity of C_3 and C_4 plants. Oecologia 44: 68–74

Yelle S, Beeson RC, Trudel MJ and Gosselin A (1989) Acclimation of two tomato species to high atmospheric CO_2. II. Ribulose 1,5-bisphosphate carboxylase oxygenase and phosphoenolpyruvate carboxylase. Plant Physiol 90: 1473–1477